Vol. 4, Nos. 4-6 April-June 1928 No. 43-45

Bulletin of the Agricultural Chemical Society of Japan.

CONTENTS

Published by the
Agricultural Chemical Society of Japan.
c/o Faculty of Agriculture, Tokyo Imperial University.

Single Copy (Postage inclusive) :- ¥ 0.35
Annual Subscription (12 numbers) :- ¥ 3.50

The Agricultural Chemical Society of Japan.

President : Keizirō Aso

The Council of the Agr. Chem. Soc. of Japan has decided to publish English Abstract of those papers appearing in the Journal in a separate form in order to facilitate the circulation in foreign countries.

Bulletin of the Agr. Chem. Soc. of Japan is published for this purpose from May 1926 monthly. The numbering begins with Vol. 2, No. 5. The earlier parts are represented by the English abstracts published in the Journal annexed to the Japanese texts.

The articles to be appeared in the Bulletin must be concise, supplied with experimental methods and data and understandable, without specially referring to the Japanese texts. It ought, however, not exceed four printed pages as a rule. Any longer articles may be accepted according to the decision of the Council, with or without charge for exceeding pages.

Journal of the Agr. Chem. Soc. of Japan will be published in Japanese as formerly. Those desiring the detailed information of the articles appeared in the Bulletin may look for in the Journal of the same Number or the same Volume.

Editor : Keizirō Aso

Associate Editors : Kakuji Gotō and Yoshihiko Matsuyama.

ON THE PROTEIN OF THE TUBER OF YAM (DIOSCORIA)

I. ON THE GENERAL PROPERTIES OF THE PROTEIN OF THE TUBER OF YAM.

By

TEIZO TAKAHASHI

(*Gifu Agricultural College, Gifu, Japan*)

(Résumé)

(Received Jan. 5th, 1928.)

The protein which is contained in the tuber of Yam (Dioscoria) is known as a plant glycoprotein. But the investigation on which is very few because of the difficulty in preparing a sufficient ambunt for the study. Mucin, an animal glycoprotein contains glucosamin and other organic compounds such as glucose, glucuronic acid, acetic acid and sulphuric acid, and these substances constitute a complex compound called "Mucoitinschwefelsäure" or "Hyaloidin". But we know nothing whether the protein of the tuber of Yam contains such a complex compound or any reducing substance among the chain of amino acid which build up the protein. The author prepared the protein of the tuber of Yam of two species according to the method of Ishii (Bulletin of the Coll. of Agri. Tokyo Imp. Univ. **2**, 97—100, 1894) and made elementary analysis and quantitative estimation of amino acids by Van Slyke's method, and further, estimation of amino nitrogen and reducing substance which are produced by the hydrolysis with 3% HCl. (See Tables below.) From these results it may be noticed that these proteins have a small quantity of sulphur when compared with animal glycoprotein. And by the hydrolysis with 3% HCl, the reducing substance is produced rapidly and reaches the maximum quantity in a short time while the amino nitrogen produced very gradually. So, it may be said that the protein of the tuber of Yam lacks such a complex compound as "Mucoitinschwefelsäure" and has a special compound, which, by the hydrolysis with 3% HCl, produces reducing substance. Moreover, it is noticeable that these proteins are rich in the amount of diamino acids and especially of tryptophan. The author is now studying the complex compound which produces the reducing substance after hydrolysis with dil. mineral acid.

Elementary compositions of the protein of the tuber of Yam (Dioscoria)

Name of species	C	N	H	S	O
Ichinen-imo	49.81%	14.45%	7.04%	0.48%	28.22%
Nagaimo	50.45	14.08	7.53	0.40	27.54

Quantitative estimation of amino acids.

Protein of the tuber of "Nagaimo"

(moisture and ash free substance)

	A	B	Average	Total N is 100
Total N	14.08	14.08	14.08	100.00
20% HCl insoluble N	0.19	0.19	0.19	1.35
20% HCl soluble N	13.89	13.89	13.89	98.65
Ammonia N	0.53	0.93	0.74	5.26
Humin N	0.10	0.09	0.10	0.71
Diamino N	4.00	3.71	3.86	27.41
Amino N	1.74	1.74	1.74	12.36
Non-amino N	2.26	1.97	2.12	15.06
Arginin N	2.29	2.17	2.23	15.06
Histidin N	0.81	0.51	0.66	4.69
Cystin N	0.09	0.09	0.09	0.64
Lysin N	0.81	0.94	0.88	6.25
Monoamino N	9.26	9.16	9.21	65 27

It contains 2.54% tryptophan (estimated by the method of C. May and E. Rosa: Jour. Biol. Chem. 54, 213—6, 1922, modified by Matsuyama; Journ Agric. Tokyo. 44, 379.)

Protein of the tuber of "Ichinen-imo"

(moisture and ash free substance)

	A	B	average	total N is 100
Total N	14.45	14.45	14.45	100.00
20% HCl insoluble N	0.17	0.17	0.17	1.18
20% HCl soluble N	14.28	14.28	14.28	98.82
Ammonia N	0.67	0.92	0.80	5.54
Humin N	0.08	0.08	0.08	0.55
Diamino N	4.03	3.85	3.94	27.27
Amino N	1.84	1.64	1.74	12.04
Non-amino N	2.19	2.21	2.20	14.22
Arginin N	2.23	2.27	2.25	15.57
Histidin N	0.78	0.77	0.78	5.40
Cystin N	0.08	0.08	0.08	0.55
Lysin N	0.94	0.73	0.84	5.81
Monoamino N	9.50	9.43	9 47	65.46

It contains 2.45% tryptophan.

Quantitative estimation of amino nitrogen and reducing substance produced by the hydrolysis with 3% HCl.

Protein of the tuber of "Nagaimo"

Th (min.)	Ratio of reducing subst.	Ratio of amino N.
10	21.0	20.0
30	84.0	34.6
60	98.2	44.9
90	100.0	57.4
120	86.8	63.0
720	75.4	91.6

480 (20% HCl)	0	100.0

Protein of the tuber "Ichinen-imo"

10	38.7	19.2
30	93.5	27.3
60	100.0	49.0
120	100.0	61.9
720	92.9	88.4
480 (20% HCl)	0	100.0

ON THE CHANGE OF BARLEY PROTEINS.

I. THE CHANGE OF PROTEINS IN PRESERVING.

By

Eiji Takahashi and Kiyoshi Shirahama.

(Received Jan. 16th, 1928.)

The quantities of proteins soluble in water, brine water, alcohol and alkaline solution were determined of the naked barley immediate after crops and of the same seeds preserved one year.

The water soluble proteins were further investigated determining amino and peptide nitrogen using trichloracetic acid and tungstic acid, referring to the Hiller and Van Slyke's study. The results were as following table.

Changing of Various Proteins in Preserving

(Sample 10g.)

	Increase		Decrease	
Water soluble N	—	—	8.4mg.	21.3%
Albumin N	—	—	3.6″	21.6″
Albumose and Peptone N	—	—	4.3″	51.8″
Amino acid and other N	—	—	0.4″	2.8″
NaCl soluble N	1.4mg.	4.5%	—	—
Alcohol soluble N	11.3″	24.0%	—	—
Alkali soluble N	4.1″	4.9″	—	—
Insoluble N	—	—	8.4″	12.0″
Total	16.8mg	33.4%	16.8mg.	33.3%

The results appear that a reserve protein such as hordein and glutelin are newly formed in preserving, especially hordein, we observe, the remarkable increase. These increased proteins, of course, must be derived from water soluble proteins such as albumin, albumose and peptone or insoluble proteins.

Amino acid and others it seems, take only a little part in these formation.

Also, hordein A and B which was already reported by the same authors, are separated individually and compared with yields of them. The result shows that A is distinctly decreased while B is greatly increased its amount.

II. THE CHANGE OF PROTEINS IN THE GROWTH OF MALTS.

The naked barley was kept in a thermostat of 25—27°C, after it was soaked in water for 24 hours.

At the intervals of 24, 48, 72 and 120 hours, the malt was taken out, dried for 12 hours in 60°C, and ground to flour by a stone mill. On the other hand, the original barley flour was prepared for a control.

Of the malt's flour at intervals of growing, five kinds of proteins and the consumed amount of organic matters were measured as reported in the previous paper.

A great change of reserve proteins such as hordein and glutelin takes place whithin 72 hours: hordein and glutelin change to the water soluble and insoluble proteins, especially the former increasing markedly, attains maximum at about 72 hours. At a early time of malt's growth, hordein take a active part earlier than glutelin and change to water solubles and insolubles the former are in most part albumoses and peptones.

As hordein underwent changing, glutelin becomes active and changes to the same substances as before one, but the water solubles being in this case mostly albumins.

At the interval of 72—120 hours, glutelin is increased in spite of the other soluble and insoluble are all decreased except the water soluble which showed a little increase.

It is perhaps from the fact that the increase of amino acid and others exceeded the decrease of the albumins, albumoses and peptones.

Hordein A and B were also separated from the malts of 24, 48 and 72 hours and their yields were compared with. The results show that A and B decrease almost parallel in the course of growing, while B almost disappears after 72 hours.

ON THE INFLUENCE OF THE ALKALINITY OF THE ASH OF FOOD UPON THE GROWTH OF ANIMALS.

By

Rinjiro Sasaki

(*From the Agricultural chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University*)

(Received Jan. 30th., 1928.)

I. ON THE ALKALINITY OF THE ASH OF FOOD.

It has been established beyond question that the mineral matter of food is an indispensable nutrient as well as proteins and vitamins. It is considered to be convenient, at present, in almost all cases of discussions about nutrition and food–chemistry, to refer the mineral matter to the ash of food.

The definition of the so–called ash on the analysis of food, however, is now being given in various ways and expressions, and without showing any decisive explanation of its meaning. The writer took into consideration many investigations concerning this problem and proposed to define the ash on the analysis of food as follhws :

Substance, which leaves acidic ashes, is burnt to ashes with the addition of an adequate definite quantity of alkali in the form of sodium carbonate. After it is burnt completely, the added alkali must be subtracted from the ash as the form of sodium oxide, and besides, charcoal, carbon dioxide and sandy matters with which the sample is contaminated.

If the substance does not produce any acidic ashes, there is no necessity to add the alkall before burning and accordingly to subtract it from the ashes. The ash thus obtained is marked as the true ash.

Not only individual elements of ashes, but the proportion of the acid-forming and the base–forming elements of ashes also is an important factor of food which should be taken into consideration in diseussing the nutrition of animals or in judging any special properties of foods such as adulteration.

The proportion of these elements is arranged for the alkalinity of ashes, which is an algebraic addition of millival of anion and cation.

The writer investigated to determine what constituents of ashes were to be calculated as factors of alkalinity and cited the true alkalinity (Berg's) and the physiological alkalinity (Pfyl's) as the most rational alkalinity. The writer also modified the Pfyl's method of titration of the methyl orange alkalinity and invented a new process of titration to determine the above two alkalinities

as easily and exactly as possible. The process is as follows:

An adequate quantity (m grm. or 1 ccm.) of sample that corresponds to about 0.1grm. of ashes is burnt to ashes in the muffle furnace. An adequate quantity of N/10 Na_2CO_3 (1N, a ccm.) is added to the substance before burning. The quantity of soda which is required for burning to ashes varies with the contents of phosphates, calcium and magnesium salts, proteins, lecithin and so on.

After burnt, it is rubbed with a little of water, and a few drops of 30% pure hydrogen peroxide is added. It is evaporated to dryness on the water bath and then burnt until free from carbon at a low heat, not to exceed dull dull redness. The above procedure is repeated until the ash is white or grayish white.

The ash thus obtained is rubbed to mud with a little of water, and a little excess of N/10 HCl (1N, S ccm.) is added to the same. The solution is transferred to a Erlenmeyer's flask with the undissolved residue, and gently boiled for 15 minutes.

On cooling, it is titrated with N/10 NaOH (1N, b ccm.) using phenolphthalein as an indicator.

Then 40% $CaCl_2$ is added to the solution in the flask and boiled for 3 minutes. After cooled, the acid liberated is again titrated with N/10 NaOH (1 N, c ccm.) using the sama indicator as the above.

The quantity of acid and alkali used in the titration is converted into the quantity of normal solution and the alkalinity is calculated for 100grm. of dry matter or 1000ccm. of liquid as follows:

$$\text{Physiological Alkalinity} = \frac{S-a-b}{m} .100 \text{ millival}$$

$$\text{True Alkalinity} = \frac{S-a-b-c}{m} .100 \text{ millival}$$

The writer examined that the above method may be applied to salts of organic acids and phosphates, and also studied the relation between this method and the Pfyl's method. The effects of silicates, iron, aluminium and manganese upon the alkalinity were determined, and the method of correction being investigated at the same time.

II. ON THE INFLUENCE OF THE ALKALINITY OF THE ASH OF FOOD UPON THE GROWTH OF ANIMALS.

The influence of the true and the physiological alkalinity of ashes of food upon the growth of animals was studied with the following results.

Twenty two albino rats were used in the experiment. The ration consisted of a mixture of polished rice, casein, takuwan-zuke (a japanese fermented pickles), lard, cod-liver oil, oryzanin (vitamin B) and various salt mixture.

All of the ration used in this experiment were uniform for the proportion of protein, carbohydrate, fat and vitamins, and the amount provided was regulated so as to furnish sufficiently the nutrients required for growing rats. If there were significant difference among the growth of animals on these rations, its difference of nutritive value of ration would have been attributed to the difference of the property of mineral matters.

The ration the physiological alkalinity of which was negative, was not sufficient to enable the young animals to make normal growth. The ration the true alkalininity of which was positive, was sufficient to enable the young animals to make normal growth. But on the other hand, the ration the difference of the two alkalinities of which was too great, indicated the fact that there was an excess in the amount of the phosphorus, one of its nutritive constituents, as compared to that of calcium.

In such case, the writer found that alkali was ineffective for neutralizing, and that it must be relied upon the calcium for neutralization.

Also by the degree of the difference of two alkalinities, it is able to measure out the content of phosphorus in the ration. For that reason, it may be useful for determining the necessity of calcium.

So far as could be measured by general observations, weights, and gross symptoms, the supply of available calcium is found to be already required for neutralization when the difference of two alkalinities is at least 9–10 millival in the case of positive physiological alkalinity, and at 3–5 millival in the case of negative physiological alkalinity.

It was reported as the result in this experiment that polished rice and takuwan-zuke were deficient in calcium.

It is a well known fact that grains or their byproducts are not sufficient for the growth of young animals. One of the chief reasons of such deficiency may due to the fact that the amount of phosphorus containd in grains and their byproducts too much exceeded that of the calcium, and that consequently the ash is strongly acidic. For neutralization of such negative alkalinity, alkali salts of organic acids are ineffective, but only calcium salts in general are effective.

When grains or their byproducts are supplied with hay, leaves or stems of vegetables, the total nutrients are regulated so as to furnish a complete ration. Such effect of supplement is dependent upon calcium, besides on positive alkalinity rather than on potassium. For supplying such deficiency, no significant effect is to be expected at all without calcium salts of organic acids.

As above shown, it appears evident that, the relative amounts of calcium and phosphorus present in a certain ration may be of significance to the

developement of young animals, and also that both of the true alkalinity and the physiological alkalinity of ashes of rations are useful in general for determining nutritive values of various foods.

III. ON THE INFLUENCE OF THE ALKALINITY OF THE ASH OF FOOD UPON CALCIUM AND PHOSPHORUS IN THE BODIES OF ANIMALS.

It has been reported, in the previous paper, that alkalinities of the ashes of foods in general were to be looked upon as an important factor in the determination of their nutritive values. The investigation reported in this paper embodies the results of the writer's attempts to examine the influences of alkalinities of the ashes of various foods upon the calcium and phosphorus of the bones of animals.

In the experiment, the comparison began with eight albino rats, weighing from 30 to 50grm. each; they were divided into three groups. Each of the group AA and AC contains two rats and the group AB contains four rats.

The alkalinities of the three rations used in the experiment descrived in this paper are given in Table I.

TABLE I.

The Alkalinity of the Ash of Rations.

Rations.	In dry matter.	
	True alkalinity (m.v.)	Physiological alkalinity (m v.)
AA	(+) 0.51	(+) 6.60
AB	(−) 9.58	(−) 3.41
AC	(−) 11.96	(−) 3.38

Ration AA provided a complete supply of nutrients. The rats on that ration were used as the control of normal growth for comparison. Ration AB contained all constituents in sufficient quantity except that there was some deficiency in calcium, besides that the reaction of the ash was not normal. Ration AC provided also an ample supply of nutrients, but the reaction of the ashes is negative. The animals were experimented on giving free access to distilled water and to the desired ration.

It will be seen from Chart that the growth of the rats on Ration AA was almost normal but the rats on Ration AB and AC could not grow so large as the group AA.

From these trials it appears that Ration AA is adequate for supporting growth, although Rations AB and AC are not satisfactory for the young rats probably owing to the bad reaction of the ashes.

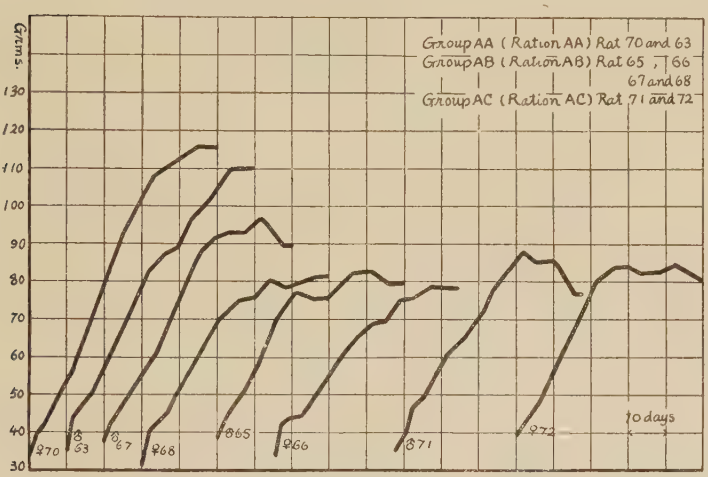
The influence of alkalinity of ashes of rations upon the growth of bones was investigated by determining their contents of calcium and phosphorus.

At the fiftieth day from the beginning of the experiment, all animals in the experiment were analyzed for determination of the content of calcium and phosphorus. The results and calculations are given in Table II.

TABLE II.

The Contents of Calcium and Phosphorus in the Femur

Rats	Ca (%)		P (%)		P:Ca	
	♂	♀	♂	♀	♂	♀
Group AA	7.00	7.07	5.00	5.00	0.71	0.71
" AB	4.07—5.50	4.24—5.00	2.57—3.70	2.80—2.90	2.63—0.67	0.58—0.66
" AC	6.00	6.42	4.00	3.83	0.67	0.67



From Table II it can be seen that, the lack of normal growth of the bones was due to the negative alkalinity of the ashes of the rations. The negative alkalinities of the ashes exerted the bad influence upon the retention of phosphorus in the bones and the decrease of the retention of phosphorus also lead to a decrease in the storage of calcium.

It was noted that the relation of calcium and phosphorus in the skins was adversely affected, quite contrary to the bones, and the retention of phosphorus here was better than it was the case with the bones.

TABLE III.

The Contents of Calcium and Phosphorus in the Skin

Rats	Ca (%)		P (%)		P:Ca	
	♂	♀	♂	♀	♂	♀
Group AA	0.17	0.18	0.20	0.20	1.16	1.11
" AB	0.13	0.11—0.12	0.18—0.19	0.18—0.19	1.43—1.54	1.57—1.62
" AC	0.18	0.16	0.27	0.24	1.50	1.45

The relation of the two elements in the bodies exclusive of alimentary canals is to be explained by Table IV.

The data in the Table IV represent the fact that, a greater proportion of phosphorus is contained as compared with calcium in the bodies of the

rats on the ration, the alkalinity of the ashes of which is negative than in the case of the rats on the ration, the alkalinity of the ashes of which is positive. The fact is more significant in the ration which lacks in calcium in addition to its ashes being negative in alkalinity.

TABLE IV..

The Contents of Calcium and Phosphorus in the Body Exclusive of Alimentary Canal

Rats	Ca (%)		P (%)		P:Ca	
	♂	♀	♂	♀	♂	♀
Group AA	0.67	0.71	0.51	0·52	0.76	0.73
〃 AB	0.52—0.53	0.52—0.55	0.42—0.46	0.37—0.49	0.81—0.87	0.70—0.90
〃 AC	0.69	0.66	0.58	0.59	0.85	0.87

Nextly the bodies exclusive skins and alimentary canals indicate the following data. It can be seen from Table V that, a greater proportion of phosphorus is contained as compared with calcium in the bodies exclusive skins and alimentary canals of the rats on the ration the alkalinity of which is negative than in the case of the rats on the control ration. The content of calcium in the rats on Ration AC, however, only slightly differs from that in the case with Ration AA. It may, then, be possible that, the ratio between phosphorus and calcium in muscles is opposite to that in bones.

TABLE V.

The Contents of Calcium and Phosphorus in the Body Exclusive of Skin and Alimentary Canal

Rats	Ca (%)		P (%)		P:Ca	
	♂	♀	♂	♀	♂	♀
Group AA	0.76	0.80	0.57	0.53	0.74	0.72
〃 AB	0.58—0.60	0.60—0.64	0.46—0.51	0.40—0.56	0.79—0.84	0.67—0.88
〃 AC	0.78	0.75	0.62	0.61	0.80	0.82

The rats on Ration AB contains less calcium in all respects than those on AA and AC, but the ratio between calcium and phosphorus in the body of the former is the same as those of the latter.

From these results, it appears that the utilization of phosphorus of rations in animal bodies is not so much affected by negative alkalinity of ashes as calcium, but that of calcium is adversely affected with bad influence.

This fact is enough to constitute an unquestionable demonstration that the alkalinity of ashes of rations is important for supporting healthy developement of young animals. The animal can not grow normal due to imperfect calcification of bones on rations the ashes of which are of negative alkalinity. (Author's abstract)

ORIGINAL PAPERS

(I) On the Alkalinity of the Ash of Food by R. Sasaki, Jour. Agr. Chem. Soc. of Japan, II-6 (1926), 428-446.

(II) On the Influence of the Alkalinity of the Ash of Food upon the Growth of Animals by R. Sasaki, Jour. Agr. Chem. Soc. of Japan, II-11 (1926), 788-805.

(III) On the Influence of the Alkalinity of the Ash of Food upon Calcium and Phosphorus in the Body of Animals by R. Sasaki, Jour. Agr. Chem. Soc. of Japan, III-11 (1927), 1171-1182.

ON POLYNEURITIS OF PIGEON AND ALBINO-RAT DUE TO THE DEFICIENCY OF ORYZANIN (VITAMIN-B).

By

MOTOE IWATA.

(Received Feb. 4th., 1928)

From the consideration on the known polyneuritis-like diseases of the pigeon and the observation of the author on polyneuritis, it has been suggested that various symptoms of polyneuritis from B-avitaminosis may be attributable to the disturbance of function in animal's central nervous system, and so he tested the effect of puncture throughout the different parts of the brain of pigeon. It has been found that the allied phenomenon develops only when the medulla oblongate is stimulated. By skillful punctures, several types of symptom in B-avitaminosis could be made to develop by means of the operation, and also he recognized that the effect is connected with the deficiency of Oryzanin. The recognition of the relation between the result of the operation and the deficiency of Oryzanin is of fair interest.

On investigating the rôle of medulla oblongata with pharmacological methods, it was found that it was temporarily afflicted with such spasmodic conditions as shown in B-avitaminosis by about 0.06g. of furfural even in healthy pigeon. By giving either by subcutaneous injection or by the mouth, 0.02-0.04g. of furfural per day, which amount could not affect the healthy pigeon at all, to the pigeon which is being induced to the deficiency of Oryzanin, a temporary convulsion was induced after several days, showing a gradual increase of the susceptibility to furfural, and the time of convulsion was prolonged day by day until at last the condition lasts for few days and the pigeon dies. However, if one dose of Oryzanin is given to any of the

cases in these conditions, the animal is cured of the disease so perfectly that the effect of furfural, even in larger amounts, cannot be recognized. By decreasing the amount of furfural, in association with the increase of the degree of the deficiency of Oryzanin, he found that a pigeon can be affected with 0.002–0.003g. of furfural and that this effect can be offset by giving a small amount (0.5c.c.) of Oryzanin on market. Also a nearly analogous result was obtained with albino–rats.

Microscopical examinations of medulla oblongata of the animals showing convulsion from the deficiency of Oryzanin indicated that no notable change takes place in this part of the albino–rat. In the case of pigeon, however, many small lesions of perivascular and local forms have been found in the medulla oblongata and other parts of the brain. This fact was proved in all of 11 cases made for this experiment. This changes are to be regard as morphological expressions of the injury of medulla oblongata.

The author also has found that such a substance which has a function something like the physiological behaviour of furfural can be obtained by boiling carbohydrates, for example, glucose, arabic gum, starch, et., with 5–10% of hydrochloric acid for 7–16 hours. This substance may probably be one of the furfural series, such as furfural, oxy–methyl–furfural and so on. From the fact that glycuronic acid is detected in urine by colour reaction even in the slightest nutritive disturbance which is associated with heightening of intestinal putrefaction, it may be suggested, though there is no positive proof, that a furfural–like substance may be produced by the nutritive disturbance due to the deficiency of Oryzanin, and that such a substance may be an affecting factor in polyneuritis.

Therefore it may be stated that some conversions take place in the case of the deficiency of Oryzanin through the all parts of medulla oblongata as in other nervous system, and the functional disturbance involved in medulla oblongata may be an important factor in B–avitaminosis. These changes make the affected parts so extraordinarily sensitive that the general symptoms convulsion, paralysis and so on, through all parts of the animal–body may be produced by means of even a slightest impulse, and finally it suffers death. Hence, the fatal effect in B–avitaminosis seems to depend in a large measure upon the disturbance of medulla centers directly connected with the life.

The author wishes to express his cordial thanks to Prof. Dr. U. Suzuki and Dr. W. Nakahara for their kind remarks during the work, and also to Mr. N. Takeshita, the Veterinary Officer, who kindly supplied him with many valuable samples.

STUDIES ON ACIDS FORMED BY RHIZOPUS SPECIES. PART VII.

FORMATION OF FUMARIC, SUCCINIC AND FORMIC ACIDS FROM ACETIC ACID, FREE FROM NITROGENOUS MATTERS.

By

Teizo Takahashi and Toshinobu Asai.

(Received Feb., 13th., 1928)

In the fourth communication[1] on this subject the authors have mentioned the formation of succinic acid from acetic acid, in its calcium salt, by this fungus, and in the sixth report[2] the other acids such as malic, lactic, fumaric and formic acids were added as the products of fermentation from the same carbon source by the same fungus.

To avoid the contribution of carbon source as the nourishment of fungus or to gain the maximum yield of the products from the same quantity of the said source, authors devised to immerse the fungus growth, which was cultured in advance in a common medium, into the special ones which devoid of or free from nitrogenous matter. This special medium contained acetic acid and its salt as the carbon source, beside some kinds of phosphates, which may be regarded as buffers as in the research of enzyme. Thus, the fungus *a priori* is almost impossible to make a growth whatsoever.

The results are tabulated below :–

	Substances.	Duration of immersion	Formic acid formed	Succinic acid formed	Malic acid formed	Lactic acid formed	Formic acid formed	Acetic acid remaind
			(g.)	(g.)	(g.)	(g.)	(g.)	(g.)
1).	Fungus + acetic acid, its salt + phosphate solut. 200c.c.	10	0.1016	0.1027	+	+	0.0172	0.5321
2).	Do	20	0.2160	0.1587	+	+	0.0121	0.3945
3).	Do, but boiled 2 hours.	20	0.0092	Trace	–	–	–	2.0340
4).	Fungus + distilled water + phosphate.	20	–	–	–	–	–	–

Remarks :– In the control case viz. the fourth experiment in the table a trace of acids was perceived, namely non–volatile acid corresponding to 0.5c.c. of n/10 NaOH and volatile acid representable by 0.6c.c. of n/10 NaOH.

From the table stated and reminding of our former papers,[3), 4)] we could not overlook the condition of the formation of succinic acid by this fungus from acetic acid. In one case, it was formed in some noticeable quantities,

while in other case it was just a trace and the difference of the conditions between two cases was, most likely, to be looked upon as the difference of the duration of cultivation. In present research, the amounts of succinic acid formed were governed by the length of times of cultivation, the longer the more and *vice versa*, and in the meanwhile the decrease of the quantity of acetic acid in the medium runs quite parallel to the increase of succinic acid. The same was also observable in the formation of fumaric acid.

However, the amount of malic acid formed was just a trace, in behalf of deficiency of the length of immersion.

These facts favour for the substanciating our equations concerning the mechanisms of the formation of these acids.

If we allow the growth to stand more longer in immersed state, malic acid may come forth in more noticeable amount, in certain period of time in as much as malic acid again be decomposed into fumaric acid and others.[5]

Notwithstanding the condition of formation of succinic acid goes another-way just stated, there must exist some special enzyme to synthesise the acid from acetic acid, linking two molecules of the latter acid. The existence of such enzyme was already assumed by Thumberg (1911),[6] although lucking of its realisation.

The origin of the succinic acid formed in fermentation by yeast has been traced by Ehrlich (1909) to the alcoholic fermentation of the amino-acids. Euler's view[7] that succinic acid be formed from acetaldehyde or acetic acid was proved by Grey with Bac. lactis aerogenes. Whereas, one of us[8] co-worked with K. Sakaguchi has affirmed the formation of succinic acid, from other source than nitrogenous substances, likewise in present case viz. by Rhizopus species the same was confirmed in a more strict sense.

The formation of formic acid from acetic acid is a delinking process of carbon atom like that of tartaric acid from gluconic acid and its change may be representable as follows :–

$$2CH_3\cdot COOH + 2O_2 = 2H\cdot COOH + 2CO_2 + 2H_2.$$

If such is the case and referred together with the former papers, the mechanism of the formation of these organic acids from glucose may be shown as follows :–

I). $2\ \begin{matrix} COH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH_2\cdot OH \end{matrix} + O_2 = 2\ \begin{matrix} COOH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH\cdot OH \\ |\\ CH_2\cdot OH \end{matrix}$

II) $2\ \begin{matrix}\mathrm{COOH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH_2{\cdot}OH}\end{matrix} + O_2 = 2\ \begin{matrix}\mathrm{COOH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{COOH}\end{matrix} + 2\ CH_3COH + 2\ H_2O$

III) $\begin{matrix}\mathrm{COOH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{CH{\cdot}OH}\\ \mathrm{COOH}\end{matrix} = \begin{matrix}\mathrm{CH_2{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix} + O_2$

IV) $2\ \begin{matrix}\mathrm{CH_2COOH}\\ \mathrm{CH_2COOH}\end{matrix} + O_2 = 2\ \begin{matrix}\mathrm{CH{\cdot}OH{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix}$

V) $2\ \begin{matrix}\mathrm{CH{\cdot}OH{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix} = 2\ \begin{matrix}\mathrm{CH_2{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix} + H_2$

VI) $\begin{matrix}\mathrm{CH_2{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix} = \begin{matrix}\mathrm{CH{\cdot}COOH}\\ \|\\ \mathrm{CH{\cdot}COOH}\end{matrix} + H_2$

VII) $\begin{matrix}\mathrm{CH{\cdot}COOH}\\ \|\\ \mathrm{CH{\cdot}COOH}\end{matrix} + \begin{matrix}\mathrm{H}\\ |\\ \mathrm{OH}\end{matrix} = \begin{matrix}\mathrm{CH_3}\\ |\\ \mathrm{CH{\cdot}OH}\\ |\\ \mathrm{COOH}\end{matrix} + CO_2$

VIII) $2\ CH_3{\cdot}COH + O_2 = 2\ CH_3{\cdot}COOH.$

IX) $2\ CH_3{\cdot}COOH + 2\ O_2 = 2\ H{\cdot}COOH + 2\ CO_2 + 2\ H_2$

X) $2\ CH_3{\cdot}COOH = \begin{matrix}\mathrm{CH_2{\cdot}COOH}\\ \mathrm{CH_2{\cdot}COOH}\end{matrix} + H_2$

Experimental.

Rhizopus sp. used was the same one as in many previous papers viz. Rhiz. G. 34 Yamazaki.

To koji–extract, the density of 10°B, was seeded with the fungus, and held at 25–30°C during 10 days. At the end of the cultivation, the nutrient fluid was decanted off, and thereupon the steril water was introduced over the growth repeatedly to wash the culture to free from sugar, acids and alcohol. To such an unique growth, solution mentioned below was added :– to 200c.c. of solution 2g. of the growth.

K–acetate	2g.	$P_H = 5.2$	Contents of acetic acid 1.423g. in 100c.c
Acetic acid	0.2g.		
n/15 KH_2PO_4	9c.c.		
n/15 Na_2HPO_4	1c.c.		
Water	90c c.		

The nature of the solution, thus far, is almost identical with those which have been used in the researches of the enzyme, with only difference of absence of toluol in this solution.

The Detection and Identification of Products of Fermentation

Aldehyde and alcohol :

The neutralisation of the solution freed from the fungus growth, with sulphuric acid was conducted, prior to the distillation ; for the solutions 1) and 2) in the table reacted alkaline. The first distillate gave reaction for aldehyde by Schiff's decolourized fuchsin solution. It gave iodoform when warmed gently to 45–50°C, in presence of iodine and soda solution.

Volatile acids :

Steam distillation was conducted with the solution stated above, which was acidified somewhat in excess with H_2SO_4 in presence of tropäoline OO as an indicator, prior to the distillation.

The distillate reduced both aqueous $HgCl_2$ and ammoniacal $AgNO_3$ solutions, showing the association of formic acid in it.

To determine quantitatively formic acid, one half of the distillate was taken and thereupon aqueous mixtures of $HgCl_2$ and Na–acetate was poured into allowing to be reduced the former salt into Hg_2Cl_2 after Porter–Kuyssen's method. Another half of it was used for the quantitative determination of acetic acid, which remained in solution when we oxydised formic acid by the mixture of K–bichromate and sulphuric acid. Acetic acid thus remained unaltered, was distilled prior to the titration of it by n/10 NaOH solution.

Non– volatile acids :

The residue of the steam distillation was extracted with ether as stated several times in our previous papers. The crystals of fumaric acid came forth after evaporation of etherial extract, giving characteristic colour reaction by resorcin or β–naphtol in presence of sulphuric acid.[8] The fluidal part gave colour reactions specific to succinic acid (resorcin and sulphuric acid)[9] and malic acid (Denigés reaction,[10] reduction of palladium chloride[11] and Hopkin's reaction[12]). β–Naphthol gave slight dark colouration in presence of sulphuric acid,[13] specific to lactic acid and thereby we could quite safely to assume the occurrence of this acid in the product keeping born in mind the positive evidence of Hopkin's reaction :

Identification of fumaric and succinic acid :

Fumaric acid :– m. p.		284°C (in sealed tube, not corrected).	
$C_4H_4O_4$.	Substance taken	Number of titration.	
	0.0964g.	Found.	16.0c.c. n/10 NaOH.
		Cal.	16.1c c. n/10 NaOH.
Ag–fumarate.	Substance taken		
$Ag_2C_4H_2O_4$	0.1928g.		
AgCl	0.1674g.	Ag. 0.1260g.	Found. 65.35%
			Cal. 65.43%
Succinic acid :– m. p.		183°C (not corrected).	
$C_4H_6O_4$.	Substance taken	Number of titration.	
	0.0751g.	Found.	12.0c.c. n/10 NaOH.
		Cal.	12.0c.c. n/10 NaOH.

Ag-succinate.	Substance taken			
$Ag_2C_4H_4O_4$	0.1630g.			
AgCl	0.1406g.	Ag. 0.1058g.	Found.	64.90%
			Cal.	65.02%

(This paper is read in the meeting of this society held on Dec. 1927.)

1) Takahashi and Asai. Bull. Agric. Chem. Soc. Japan. Vol. 3. No. 2. 1927. and J. Agric. Chem. Soc. Japan. Vol. III. No. 5. 1927.
2) Takahashi and Asai. J. Agric. Chem. Soc. Japan. Vol. III. No. 1. 1927.
3) Loco citato.
4) 〃 〃
5) Takahashi and Sakaguchi. J. Agric. Chem. Soc. Japan. Vol. III. No. 1. 1927.
6) Thumberg, Skand. Arch. phy. **25**, **37**, (1911). durch die Fermente. C. Oppenheimer. s. 1339. u. 1342.
7) Neuberg's article. Ber. 1924. **57**, 1394, Throu. Woch. f. Brau. 1924. Nr. 41. s. 223.
8) Takahashi and Sakaguchi. J. Agric. Chem. Soc. Japan. Vol. III. No. 6. 1927.
9) 10) Takahashi and Sakaguchi. J. Agric. Chem. Soc. Japan. Vol. I. No. 14. 1926.
11) Jahres. d. Pharm. **35**. (1900). 237.
12) Hilger's reaction. Zeit. f. Unters. d. Nähr.- u. Genusz. **4**, (1901). 49.
and 〃 〃 〃 〃 **2**. (1899). 795.
13) J. of ph. **35**. 308. (1907).
14) Reaction for lactic acid. Takahashi and Sakaguchi. J. Agric. Chem. Soc. Japan. Vol. I. No. 14. 1926. or. Bull. Agric. Chem. Soc. Japan. Vol. 2. No. 8.

NEW COLOUR REACTIONS OF BIOSTERIN

By

Sadayuki Hamano.

(Received Feb. 29th., 1928)

Biosterin which is prepared by K. Takahashi's method; unsaponifiable matter of cod-liver oil is purified removing the prectpitate produced in an acetonic solution cooling at -65°C and the trace of cholesterol by digitonin, gives all the colour reactions attributed to vitamin A which have been published of course, i. e. by each addition of conc. sulphuric acid, phosphoric acid, phosphorus anhydride, acid-clay, or chloride of aluminium, zinc, magnesium, titanium, arsenic, or antimony to the biosterin a blue colour appears as in the case of cod-liver oil. These reagents are all of strong dehydrating agents. But by other sorts of reagents, polyphenols or aromatic and heterocyclic amines the author found the new colour reactions of the biosterin, as follows:

(I) Phloroglucine:- To an alcoholic solution of the biosterin, a small quantity of phloroglucine and a few drops of conc. hydrochloric acid was

added (the same afterwards) a green colour appeared at first, which turned sky blue and remained unchanged for about 10 minutes, then fading in prussian blue, rose pink in the last. In the case of cod-liver oil it took some time when the blue colour appeared and at first a pink colour developed.

(2) Aniline, Xylidine, Benzidine, Phenylhydrazine, or Naphthylamine :- In the same way as (1) these amines in stead of phloroglucine produced a red colour glacial acetic acid being added also.

(3) Indole :- Dark green to brown in the last.

(4) α-Methylindole :- Sky blue colour as in the case (1).

(5) Skatole :- Yellowish brown.

(6) Pyrrole :- Green at first which turned promptly dark brown.

(7) Chinaldine :- Yellowish brown.

The limit of these colour reactions examined by phloroglucine and naphthylamine lied in $\frac{1}{600,000}$ of the biosterin. The author observed that many aliphatic and aromatic aldehydes also produced with these polyphenols or amines special colours or precipitates ; especially the reactions of furfural resembled very much those of the biosterin. On the other hand biosterin reduces Fehling's solution, ammoniacal silver nitrate, phosphomolybdic acid as already known. From these facts it is not unreasonable to infer that the biosterin may have an atomic group of carbonyl. Cholesterol does not give the above colour reactions.

In conclusion the author wishes to express his sincere thanks to Prof. Dr. U. Suzuki for his constant advices and the kind guidance throughout the whole experiment.

ON THE DIFFERENCES OF BREWING BARLEY ACCORDING TO SPECIES.

III. THE INVESTIGATIONS OF THE STARCHES.

By

Yukihiko Nakamura.

(Received March 14th., 1928)

Introduction.

Many investigations concerning the starches of the brewing barley carried out by such authors as Lintner, Wenglein, Ewers, Schubert, Schwarcz, Kastscha, Ling, Ling and Nanji and Harper were mainly connected with the

methods to detetmine quickly the starch content of its granules. No studies have been carried out on the differences of the starches of barleys according to the species. However, T. Tadokoro and his co–workers have made precise investigations on the differences of the starches of glutinous and common rices. The author has already reported some physico–chemical differences and the kinetics of enzymatic decomposition of the proteins of brewing barley according to the species. The present investigations were carried out continually on the starches prepared from the brewing barleys, Chevalier, Golden Melon and Hokudai No. 1, produced in the years of Taisho 12, 13 and 14 (1923, 1924 and 1925).

Conclusions.

1. The specific viscosities of the Chevalier starch solution was the greatest, that of the Golden Melon intermediate, and that of the Hokudai No. 1 was the smallest.

2. The saponification value of the Hokudai No. 1 starch was the greatest, that of the Chevalier intermediate, and that of the Golden Melon was the smallest.

3. According to the absorption of iodine from I–KI–solution, the Golden Melon starch absorbed the greatest quantity, the Hokudai No. 1 an intermediate quantity, while the smallest quantity was absorbed by the Chevalier starch when the concentration of iodine was low. But when the concentration was high, there was no difference according to the species.

4. The content of acetyl groups was the greatest in the Chevalier acetyl starch, intermediate in the Golden Melon acetly starch, and the smallest in the Hokudai No. 1 acetyl starch. The specific rotatory power and the melting point of the acetyl starches were just the contrary. In the chlorine substitution of acetyl starch, the quantity of chlorine substituted was the greatest in the Chevalier acetyl starch, intermediate in the Golden Melon acetyl starch, and smallest in the Hokudai No. 1 acetyl starch.

5. From the acetylation of the starches, the viscosity measurements of the starch solutions and the chlorination of the acetyl starches, it seems (1) that the molecular weight of the Chevalier starch is the greatest and also that the molecule is the most loosely composed of the three kinds of starches, (2) that the smallest and also the hardest is the Hokudai No. 1 starch, and (3) that the Golden Melon starch is situate between these two in respect to the molecular weight and the mode of composition.

PREPARATION OF FURANETHYLAMINE.

By

Teijiro Yabuta and Katsuji Kambe.

(*Agricultural Chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University*)

(Received March 31st., 1928)

Three methods of the syntheses of furanethylamine are described in the literature as follows.

1. Windaus and Dalmer[1] obtained the amine from furanpropionic acid, by converting the latter successively into the corresponding ester, hydrazide, azide and urethane, and finally by distilling the urethane mixed with calcium oxide and calcium hydroxide.

2. Asahina and Fujita[2] prepared the amine from furfural by means of the following reactions.

$$C_4H_3O\cdot CHO + ClCH_2COOC_2H_5 \xrightarrow{Na} C_4H_3O\cdot \underset{\diagup\ \diagdown}{CH\text{–}CH}\text{–}COOC_2H_5$$

$$C_4H_3O\cdot \underset{\diagup\ \diagdown}{CH\text{–}CH}\text{–}COONa + NH_2OH\cdot HCl \longrightarrow$$

$$C_4H_3O\cdot CH_2CH{=}NOH \longrightarrow C_4H_3O\cdot CH_2CH_2NH_2$$

3. L. Bouveault and A. Wahl[3] prepared the amine by the condensation of furfural with nitromethane in alcoholic solution in the presence of alkali and by the reduction of the resulting furannitroethylene at first to furanacetaldoxime with zink and acetic acid in ether solution and then to furanethylamine with sodium amalgam as the following.

$$C_4H_3O\cdot CHO + CH_3NO_2 \longrightarrow C_4H_3O\cdot CH{=}CHNO_2 \longrightarrow$$

$$C_4H_3O\cdot CH_2CH{=}NOH \longrightarrow C_4H_3O\cdot CH_2CH_2NH_2$$

By the application of Gabriel's reaction[4] the authors have succeeded in preparing furannitroethylene by the action of nitrous acid anhydride upon furanacrylic acid, which is now manufactured as preservative for "Shoyu" and obtained at a moderate price.

$$C_4H_3O\cdot CH:CHCOOH \xrightarrow{NO_2} C_4H_3O\cdot CHNO_2CHNO_2COOH$$

$$\xrightarrow{H_2O} C_4H_3O\cdot CH:CHNO_2$$

The reduction of furannitroethylene to furanacetaldoxime was found to be carried out more smoothly with aluminium amalgam than with zink and acetic

acid used by Bouveault and Wahl (loc. cit.). For the reduction of the acetoxime to the corresponding amine, the authors followed quite the same as Asahina's direction (loc. cit.).

Experimental.

Furannitroethylene.——1 pt. of finely powdered furanacrylic acid was suspended in 5 pts. of benzol (in the other case, furanacrylic acid was dissolved in hot benzene and cooled rapidly to make the crystals separate as finely as possible) and an equivalent quantity of nitrous acid anhydride was passed into the mixture, cooling with ice and salt. The resulting resinous substances were isolated, treated with water and extracted with ether. The brownish crystalline mass left on evaporation of the ether, was crystallized from petroleum ether (b. p. 40—50°) in large, lustrous, yellowish crystals, which melted at 75° and boiled 110°/4mm. These crystals were confirmed to be identical with the furannitroethylene prepared by the condensation of furfural with nitromethane. The yield was not good. (Found: N=10.04 $C_6H_5NO_3$ requires N=10.07 per cent.)

Furanacetaldoxime.——1 pt. of furannitroethylene was dissolved in 20 pts. of ether which had been saturated with water. 2 pts. of newly prepared aluminium amalgam were added to the solution, cooling in running water. The yellow colouration of the nitro-compound faded gradually in the course of the reaction. On standing overnight, it was filtered from aluminium hydroxide, mercury and aluminium amalgam remained unchange. After evaporating the ether, a brownish syrup was obtained. It mainly consisted of unstable syn-aldoxime which tasted 500 times sweeter than sugar as Asahina's observation. After a few days it changed into crystalline mass which may be mostly of the anti-aldoxime of furanacetaldehyde contaminated with the syn-compound as Asahina's suggestion. It was extracted with hot petroleum ether (b. p. 40—50°) in which the crystalline part is rather soluble. The brownish crystals which had separated on cooling was recrystallized from petroleum ether (b. p. 40—50°) as large plates, some times 3—4 cm. in length. It melted at 64° and distilled at 90—92°/4 mm. (Found: C=57.28 H=5.73 N=10.85 $C_6H_7NO_2$ requires C=57.60 H=5.60 N=11.20 per cent.)

Furanethylamine.——To 1 mol. of furanacetaldoxime in methyl alcoholic solution cooling with ice and salt was added about 3 mols. of 3% Na-amalgam. The mixture kept always slightly acidic by occasional addition of 50% acetic acid. At the end of the reaction, the methyl alcohol was evaporated from the filtrate under diminished pressure. The residual aqueous solution was extracted twice with ether. It was then made strong alkaline with sodium hydroxide and extracted again with ether several times. The second ether

extract was dried with solid potassium hydroxide, filtered and evaporated. On fractionating the residue, furanethylamine distilled at 155° under atmospheric pressure. It readily absorbed carbon dioxide and converted into the carbaminate which formed white crystals, melted at 86—87°. The yield was not good. The picrolonate was prepared from the carbaminate by adding an aqueous solution of picrolonic acid. It was crystallized from hot water in yellow prisms and decomposed at 203°. (Found: N=18.43 $C_6H_9NO \cdot C_{10}H_8N_4O_5$ requires N=18.64 per cent.)

Bibliography.

1) Windaus and Dalmer: Ber., **53**, 2304, 1920.
2) Asahina and Fujita: J. Pharm. Soc. Japan, **490**, 1084, 1922.
3) L. Bouveault and A. Wahl: Comp. rend., **135**, 41, 1902; Bull, Soc. Chim. (3) **29**. 521, 1903.
4) Gabriel: Ber., **18**. 2438, 1885.

STUDIES ON IMIDAZOL DERIVATIVES.

PART I. PREPARATION OF 4 (5)-METHYLGLYOXALINE.

By

Teijiro Yabuta and Katsuji Kambe.

(*Agricultural Chemical Laboratory, Faculty of Agriculture, Tokyo Imperial University.*)

(Received March 31st., 1928)

The preparation of 4 (5)-methylglyoxaline by the method of Windaus and Knoop has been modified in the following manner, so that instead of allowing to stand the reaction mixture at room temperature for several weeks it is heated in water bath at 50—60° for two days, and the purification is carried out by direct distillation of the raw product in a vacuum, the object in view being the simplification of the procedure for the preparation of methylglyoxaline in a large amount.

Zink hydroxide was prepared by adding 425c.c. of 28% ammonia to the solution of 1kg. of crystalline zink sulphate (corresponds to 563g. of anhydrous substance) dissolved in 8L. of water. It was at first washed with water by decantation and then on Buchner's funnel until free of sulphate. The zink hydroxide, after well drained, was dissolved in 2L. of 28% ammonia. 1.35kg. of commercial crude glucose (containing 74% of anhydrous glucose,

correspond to 1kg. of pure substance) was dissolved in it.

The solution was placed in a pressure bottle and heated in a water bath at 50—60° The reaction was found to be complete about in two days, since no more precipitate separated from the supernatant solution set apart.

The precipitate was filtered by suction and drained on porous plate yielding 320g. It was now suspended in warm water and hydrogen sulphide gas was passed to it. The filtrate together with the washing water from the zinc sulphide were evapolated under diminished pressure. On fractionating the residue, methylglyoxaline distilled at 144–146/4mm. yielding 120g.

The picrate was prepared and analysed; (found: N=22. 63 $C_4H_6N_2 \cdot C_6H_3N_3O_7$ requires N=22.51 per cent.) The nitrate melted at 115°. and the hydrochloride was extremely hygroscopic plates.

Bibriographiy.

(1) Hoppe Seyler: Ber., **4**, 346 (1871).
(2) Shützenberger: Bull. Soc. Chim., **2 25**, 289.
(3) Nencki und Sieber: Journ. für prakt. Chem., **2 24**, 498 (1881).
(4) Windaus und Knoop: Ber., **38**, 1166 (1905).
(5) Pinkus: Ber., **31**, 32 (1898).
(6) J. V. Nef: Ann. d. Chem , **335**, 254, 279 (1904).
(7) Wohl: Lippmann, Chemie der Zuckerarten, (1904) 1891
(8) Buchner: Ber., **38**, 621 (1905).
(9) Gabriel und Pinkus: Ber., **26**, 2205 (1893).
(10) Jowett und Potter: Journ. Chem. Soc., **83**, 464.
(11) Windaus: Ber., **39**, 3886 (1906).

STUDIES OF GERMINATION OF SEEDS. PART I.

TRANSFORMATION OF NITROGENOUS COMPOUNDS DURING GERMINATION OF SOYA-BEAN SEEDS.

By

Shuiku Sasaki.

(From the Biochemical Laboratory, Department of Agriculture Kyushu Imperial University.)

(Received Apr. 1st., 1928)

The present paper consists of two parts; that is, "Method of Germination" and "Analysis of nitrogenous compounds in seeds and seedlings".

A. Method of germination.

When seeds are germinated in the dark, they are usually infected by moulds or bacteria and cannot make normal growth without the use of an adequate method of sterilization. We made use of the following method to sterilize soya-bean seeds and get good result, as being harmless for seeds and yet protecting them completely from infection.

Method :– A definite amount of selected seeds is placed in a beaker containing 70 per cent alcohol, agitated several minutes and then the alcohol is poured off. The seeds are removed to a glass bottle containing 0.2 per cent mercuric chloride. The bottle is connected to a water sucker by means of a rubber tube provided with a cotton wool air filter in the middle, and evacuated and shaken for 3 or 4 minutes. Then the mercuric chloride is poured off, and the seeds are completely washed with distilled and sterilized water. The seeds are sowed in a loosely covered glass vessel, which contains silica sand and is pasted with so called "urushi paper" around its wall. The above procedure is performed in Hansen's inoculating box.

B. Analysis of nitrogenous compounds of seeds and seedlings.

Since the isolation of asparagine, in leguminous seeds germinated in the dark by Schulze,[1] many investigations concerning the mobilization of nitrogenous compounds in the course of germination are reported; nevertheless many phenomena are unexplained.

We sowed soya-bean seeds in nitrogenous compounds free silica sand and placed some in the light and others in the dark, and investigated the amounts of total N, heat coagulable protein N which was also analysed in detail by Van Slyke's method, protein N precipitated by Stutzer's method, precipitated N, and non-precipitated N, by phosphotungstic acid in the filtrate of the heat coagulable protein and free ammoniacal N. The results are as follows.

1. The amount of total N did not change during germination.
2. Protein was gradually decomposed. The decomposition was more remarkable in the seeds germinated in the dark than that in the light.
3. Heat coagulable protein was analysed by Van Slyke's method improved by Plimmer.[2] Where the total N is the same, N insoluble in concentrated hydrochloric acid was gradually increased both in the light and in the dark, and on the contrary amide N was slightly decreased. The quantitative changes of various amino N were in the limit of experimental error. Therefore intermolecular change of the protein was not observed.
4. After coagulation by heat at adequate acidity there occurred no precipitation in its filtrate by Stutzer's method.

5. In seeds and seedlings free ammonia was scarcely detected. It seems, therefore, that the urease which is contained in quantity in seeds and seedlings does not act upon urea during germination or that ammonia was converted into other compounds such as amides as soon as it was produced.
6. At first the amount of phosphotungstic acid precipitable N in the filtrate of heat coagulable protein was more than that of non-precipitable N. In the course of germination, both N increased gradually, but the rate of increase of the amount of non-precipitable N was greater than that of precipitable N.

(1) E. Schulze: Biochem. d. Pflanzen. 2 Bd. S. 256. (1920)
(2) R. H. A. Plimmer: Biochem J. Vol. 19. p. 1004. (1925)
Biochem J. Vol. 21. p. 247. (1927)

MICROBIOLOGICAL INVESTIGATION OF SOME ARABLE AND VIRGIN SOILS

By

Arao Itano and Satiyo Arakawa.

(Ohara Institute for Agricultural Research, Okayama-Ken, Japan.)

(Received Apr., 8th., 1928)

The distribution and some physiological groups of microorganisms in the volcanic soils were investigated. The soil samples of the following description were collected from Chausubara and Sakura-jima as indicated below:

Sample number.	Description.
1.	arable surface soil from Chausubara.
2.	〃 〃 〃 〃 Sakura-jima.
3.	15 cm. of top layer, dark gray colored, volcanic materials of 1914, Sakura-jima.
4.	23 cm. of 2nd layer, grayish volcanic materials mixed with gravel, Sakura-jima.
5.	61 cm of 3rd layer, dark brown sand, ibid.
6.	84 cm. of 4th layer, graish sand mixed with gravel, ibid.
7.	114 cm. of 5th layer, surface soil of arable farm, existed previous to 1914 eruption, Sakura-jima.
8.	139 cm. of 6th layer, subsurface soil, ibid.

These samples represent the arable and virgin soils of different origin and age, of which no micro-biological information is available so far as the authors are aware.

Chausubara is a newly, cultivated plantation located in Miyazayi Prefecture, Kyushu, Japan, and the soil there is said to be diluvial which was probably formed after the tertialy period. The bed of the diluvial stratum is chiefly made up of volcanogenous materials.

Sakura-jima is a volcanic island rising in the Bay of Kagoshima, Kyushu, Japan. The island suffered numerous eruption in the past and especially it became well known all over the world after the great eruption took place in 1914. The sample #2 has been the arable soil on which that famous radish, Sakura-jima Daikon, is growing at present. The samples #3-#6 inclusive were taken from each layer of profile which evidently formed four distinct layers since 1914 on top of the old arable soils represented by the samples #7 and #8. Thus the soils from Sakura-jima have comarátively recent origin and it is a great interest to find out as to the distribution and groups of microorganisms.

The following physiacl analyses besides the microbiological investigations, were carried out: moisture content, water-holding capasity, mechanical analyses, specific gravity, loss on ignition and hydrogen ion concentration.

The microbiological investigations were carried out as to: quantitative determination, the ammonifiers, the azofiers (nitrifying, nitrification and denitrification) and the cellulose fermenters. In connection with the quantitative determination such factors as chromogenesis, morpholgical as well as spore formation, Gram staining were determined on different cultures isolated.

The following summary and conclusions are given:

1. All the soils examined were slightly acid viz. $P_H < 7$.
2. The number of aerobic bacteria grown on the nitrogenous and nitrogen free media, was found to be as follows:

Medium	Microorganisms	Soil samples. #1 (thousand)	#2	#3—#6	#7
I.	Total.	8,280	7,370	430—1,246	1,480—1,760
II.	"	7,350	6,900	430—800	700—760
I.	Actinomycetes.	15.4%	14.8	0— 24.2	1.5— 5.2
	Fungi	1.3	1.2	1.8— 12.1	1.0— 1.5
	Bacteria.	83.3	84.2	63.7— 97.3	93.8—97.0
II.	Actinomycetes.	16.5%	15.1	0— 6.7	0—14.3
	Fungi.	2.6	1.3	4.3— 6.7	2.0— 7.1
	Bacteria.	80.9	83.6	86.6— 95.7	78.6—98.0

Medium { I, nitrogenous. II, nitrogen free.

3. The majority of organisms are non-pigmented.
4. Eighty four strains of organisms were isolated and most of them are

cocci or short rods and comparatively few long rods. More than half of these strains are non spore-formers. Most of them were found to be Gram positive.

5. The ammonification capacity is very feeble except in the arable soils.
6. More or less nitrification occur in all soils examined.
7. The denitrification occurs in the arable soils but none in others.
8. The fixation of nitrogen by Azotobacter was observed only in soil 2♯.
9. The fermentation of cellulose was found in the arable soils only.
10. The most striking fact is that so many organisms which are able to on the nitrogen free medium, were found in the virgin soils.

ON THE PRODUCTION OF ACETYLMETHYLCARBINOL AND 2,3- BUTYLENEGLYCOL BY MICROBES I.

MOST MICROBES SEEM TO HAVE AN ABILITY OF ACETOIN-PRODUCTION.

By

YUKIO TOMIYASU.

(From the Chemical Laboratory, Department of Agriculture Kyushu Imperial University)

(Received Apr. 9th., 1928)

The production of Acetylmethylcarbinol (acetoin) and its reduction product 2,3-butyleneglycol by various microbes (about 20 species of bacteria, 10 species of yeast, only one fungus, Mucor mucedo) has often been reported by many investigators.

As to the mode of the production of acetoin and 2,3-butyleneglycol, Neuberg stated that, by the aid of carboligase, acetaldehyde, which is formed in a nascent state as an intermediate product in the fermentation, undergoes an acyloin condensation with aldehyde which is added to the fermented liquid, and gives rise to acetoin, and the acetoin thus formed is reduced into 2,3-butyleneglycol. On the other hand, Kluyver explained this mechanism by the catalytic hydrogen-transformation theory (Wasserstoffübertragungstheorie). Kostytschew suggested that the carboligatic condensation is to be due to a specific reaction-ability of pyruvic acid. Although the latter two investigators denied the existence of carboligase, which was suggested by Neuberg as a specific enzyme being capable of condensing aldehydes, they did not absolutely

deny the enzymic formation of acetoin.

The writer also does not deny the enzymic formation. But the writer does suggest that acetoin may also be formed by the simple chemical action of nascent acetaldehyde, and that all organisms which produce acetaldehyde as an intermediate product of fermentation may be able to produce acetoin or 2,3–butyleneglycol.

The production of acetoin called the attention of many investigators as an important reaction since it is suggested as the first stage of the carbon-chain-synthesis in the organism. If it be so, it seems to be very natural to suppose that all organisms may have the ability of producing acetoin or 2,3-butyleneglycol, because all organisms have the ability of fat-synthesis.

It will not be useless, therefore, to determine whether all microbes produce these compounds or not, for the purpose of the investigation of the mechanism and the physiological significans of acetoin–production. From this point of view the writer examined the production of these compounds by Kluyver's method, and confirmed the fact that all the microbes tested produced more or less of both acetoin and 2,3–butyleneglycol or at least one of them. The results obtained are shown in the following table.

Experimental.

As medium 80c.c. of koji extract (10° Balling) was used for yeast and fungus. For bacterium 50c.c. of neutralized koji extract (10° Balling) containing 1g. of calcium carbonate was used. 2 platinum ears of microbes were inoculated to each.

TABLE.

Microbes	Acetoin	2,3–Butylene-glycol	Temp.	Duration of culture
B acidi–lactici 131	+++	+	35°	7 days
" aquatilis sulcantus	+++++	++	"	"
" aureus	++	++	"	"
" butyricus	++	+++	"	"
" centrosporus	±	++	"	"
" cereus	+++	++	"	"
" coli C	++++	not tested	"	"
" coli	+	−	"	"
" cominicus	++++	+++++	"	"
" erythrogenes	−	±	"	"
" enteritidis Gaertner	+	±	"	"
" fluorescens liquefaciens	±	+	"	"
" Granulobacter saccharobutyricum	+	±	"	"
" lactis innocuum	±	−	"	"
" Loeffler typhi	±	−	"	"
" mycoides roseus	+++	+	"	"

B. Megaterium	++	+++	35°	7
" mesentericus fuscus	++++++	++++	"	"
" mesentericus vulgatus	++++	++	"	"
" natto H.	++++++	++++++	"	"
" natto S.	++++++	++++	"	"
" neopolitans	++++	++++++	"	"
" oxalaticus	++++++	++++	"	"
" prodigiosus	++	+	"	"
" pumilus	++++	++++++	"	"
" proteus	+	++	"	"
" subtilis 106	++	+	"	"
" stutzeri	+++	+++	"	"
" typhi murium	+++	++	"	"
" vulgaris	++++	++++	"	"
Micrococcus ureae	+	−	"	"
Sarcina albida	+	−	"	"
Staphylococcus candidus	++++	+	"	"
" candicans	+	+	"	"
ureae	+	−	"	"
Spirillum rubrum	+	−	"	"
Iron bacteria ?	+++++	not tested	"	"
Azotobacter Beijerinki	+++	++	"	"
Actinomyces No. 1.	+	−	32°	21
" No. 3.	+	+	32°	21
Saccharomyces apiculatus	++	+	27°	7
" cartilaginosus	−	±	"	"
" ellipsoideus	++	−	"	"
" exiguus	±	−	"	"
" Ludwigii	±	−	"	"
" Pastorianus	++	+	"	"
" saké A. No. 3.	−	−	"	"
" shaoahing No. 1.	+	±	"	"
Beer yeast (Asahi Brewery)	±	−	"	"
Distillery Race 2	++	+	"	"
Carlsberg bottom yeast No. 1.	+	−	"	"
Johanisberg wine yeast	+	−	"	"
Zygosaccharomyces Barkeri	+	−	"	"
" Major	+++	+	"	"
" salsus	++	++	"	"
" soja A	++	+	"	"
Debaryomyces globosus	++	+	"	"
Schwanniomyces occidentalis	+	+	"	"
Pichia membranaefaciens	−	−	"	"
Willia anomala	+	+	"	"
Schizosaccharomyces octosporus	+	−	"	"
" Santawensis	+	−	"	"
Endomyces fibuliger	−	−	"	"
Mycoderma M. No. 1.	−	−	"	"
Oidium lactis	++	+	"	"

Oidium albicans	+	+	27°	7
Monilia candida	++	+	〃	〃
Monilia (California Univ.)	++	+	〃	〃
Torula B.	−	−	〃	〃
Chalara mycoderma	−	+	〃	〃
Aspergillus oryzae A.	++	−	35°	7
〃 mellius	±	−	〃	10
〃 niger	++	−	〃	7
Penicillium glaucum	−	±	〃	〃
〃 purpurgenum	++	+	〃	〃
Monascus purpureus	+	−	〃	〃
Rhizopus Delemar	++	++	〃	〃
〃 chiuniang	−	±	〃	〃
Mucor racemosus	+++	−	〃	〃
〃 alternans	++	−	〃	〃
Dematium pullulans	+	+	〃	〃
Cunninghamlls elegans	−	−	27°	〃
Citromyces Pfefferianus	++	++	35°	〃
Chlamydomucor javanicus	+	+	〃	〃
Sachsia sp.	++	+	〃	〃

Some microbes, which did not show the production of these compounds in the experiments mentioned above, were cultured in 1 litre of Hayduck's medium containing 10 per cent of fructose, at 27° for 14 days, and the detection was carried out as follows: Acetoin was detected after extracting with chloroform, and the glycol was tested in the alcohol–ether extract after condensing it in vacuum.

TABLE

	Acetoin	2,3-Butylenlglycol
Sacch. saké A. No. 3.	+++	+++++
Pichia membranaefaciens	+	++
Endomyces fibuliger	+	++
Mycoderma M. No. 1.	++	++++
Torlua B.	+	+++

Cunninghamlls elegans was tested again after cultivating it in 80c.c. of koji extract containing 0.002 per cent methyleneblue, at 27° for 7 days. This time acetoin alone was detected.

It will be seen from the results of these experiments that all microbes seem to have the ability of producing acetoin or 2,3-butyleneglycol in the fermentation of sugar. This fact forms a contribution in support of my hypothesis regarding acetoin-production.

ON THE VOLATILE CONSTITUENTS OF THE SAKÉ

By

Masakazu Yamada, Shō Ishida and Chōzaburō Kobayashi.

(Received Apr. 25, 1928)

The volatile substances in the fermentation products may be said to be quite important for they form the delicate flavours. Nevertheless in the saké, only a few—acetaldehyde, methyl alcohol, furfurol-like substance etc.—have-hitherto been verified their occurrence apart from the standpoint of ordinary analysis. The authors treated the "moto"—culture solution of saké-yeast—and also the saké and isolated following substances.

From ca. 480 L. of the moto containing rice-grains.		From 77 L. of the saké
Acetald., valerald., furfurol-like subst.		〃
Formic acid, acetic acid, cadaverine		〃
Ethyl alcohol (ca. 93%)	56381c c.	9762c.c.
Butyl alcohol (iso)	10.755g.	4.7g.
Isoamyl alcohol	81.3g.	5.8g.
Sec. butylcarbinol	5.1g.	1.6g.
Fusel oil as iso amyl alc. Remained in the ethyl alc.	9.6g.	1.0g.
Distilling residue of fusel oil		
Palmitic acid ethylester (b. p. below 24—28°)	17.0g.	6.4g.
Stearic acid ethylester (b. p. 32—33°C)	39.5g.	

Experimental.

I. The general composition of the samples.

		sp. gr.	Alc. %	T. acid %	Vol. acid %	Fusel oil %	Ester %	Ald. %
Filtrate of moto	No. 8	1.008	16.86	0.5514	0.0907	0.04	0.3234	0.00502
〃	No. 22	1.017	14.81	0.7552	0.1392	—	—	—
saké	No. 6	0.995	17.60	0.1549	0.0151	0.06	—	0.00641

II. In the case of the moto.

1. Volatile acid :–

185 liters of the filtrate were distilled in steam and the distillate was taken three times as much as the original solution. The sodium salt of the volatile acid prepared was acidulated with H_2SO_4, extracted with ether and then the ethereal extract was fractionated.

Fract: I. 85—95° A small quantity II. 95°—105° 4g. III. 105—122° 8.4g.
IV. Above 122° brown residue

Sparelingly soluble Pb salt prepared from fract. II (1.1g) :–

Sub 0.2623g. $PbSO_4$ 0.2608g. Pb (found) 68.33% (cal. for $PbC_2H_2O_4$) 69.71%

Ag salt from fract. III. :– Colorless needle

Sub. 0.2132g. AgCl 0.1800g. Ag (found) 63.54% (cal. for $C_2H_3O_2Ag$) 64.64%

2. Separation of fusel oil etc.

About 480 liters of the moto solution containing rice grains were distilled and the distillate was rectified until its boiling point reached to 80°C, while the oily layer floated over the distillate and the residual watere solution was separated or extracted with ether. Now the alcohol solution was distilled repeatedly by means of the still-head until vanilline–sulphuric acid test for fusel oil showed almost negative.

3. Base :–

After being extracted with H_2SO_4 from the oily layer and fusel oil fraction, the picrate of the base was prepared according to the ordinary process, which is yellow needle and melts at 221° (Cadaverine picrate).

4. Fusel oil :–

Fract.	b. p.	yield	Fusel oil	Ester	3,5–Dinitro-benzoate		Ph. urethan	P–nitro ph. of oxidation prod.	
					m. p.	N %	m. p.	m. p.	N %
I	49—78°	13.5c.c.	0.39g.	—	—	—	—	—	—
II	78—95	37.0g.	1.97	—	87°	—	30°	—	—
III	95—102	2 7	1.70	—	—	—	53	—	—
IV	102—107	0.8	—	—	48°	—	—	—	—
V	107—114	1.3	—	1.38%	63°	10.56 (But. 10 46)	67	111°	20.08 (But. ald. 20.30)
VI	114—120	1.1	—	7.04	—	—	—	91°	19.98
VII	120—126	1.9	—	—	—	—	—	—	—
VIII	126—132	14.5	—	0.50	57°	9.78 (Amyl. 9.93)	48°	101	19.12 (Valer. 19.00)
XI	residue	5.0	furfurol reaction is positive.				—	—	—

Oxidation test : About 1g. of each alcohol was oxidized with chromic acid mixture. II and III did not give acetone or propion aldehyde for the preparation of their p–nitrophenylhydrazones and dibenzal acetone proved to be negative, while Rimini's test for acetaldehyde was positive and so the fusel oil conteint was corrected. V, VI and VIII gave the corresponding aldehydes but the m. p. of p–nitrophenylhydrazone did not coincide with the proper one. Perhaps the fractions may yet mix a trace of other alcohols. The mixture of p–nitroph. from VI and n–butyrald.–p. nitro–ph. melts at 80°.

5. Existence of secondary butylcarbinol.

α_D^{19} of VIII is –1.28° in ethyl alcohol solution. Then the approximate content of secondary butylcarbinol in tolal amyl alcohol calculated from $\alpha_D^{20} = -5.90°$ amounts 21.67%.

6. Oil of higher boiling point.

Fract.	b. p.	yield	m. p.	Fract.	b. p.	yield	m. p.
I	80—110°	8.5g.	—	VII	275—300°	0.5g.	33°
II	110—124	1.5	—	VIII	Above 300	47.0	30
III	124—134	7.0	—		(VIII is distilled in vacuo)		
IV	134—210	1.0	14°	IX	98—110°(9mm.)	0.86	—
V	210—240	3.5	17°	X	110—165°(〃)	7.1	24—28
VI	240—275	5.5	24—26°	XI	165—184°(〃)	39.0	33°

IV–VIII does not give the derivatives of alcohol but shows a very high-saponification value. On saponification of XI 15.18% of ethyl alcohol and a fatty acid of m. p. 68° were obtained.

p–nitrobenzoate and 3–5–dinitrobenzoate of the alcohol melts each at 56° and 92° N–content of the latter is 11.56% (calc. for $C_9H_5O_6N_2$ 11.67%) 0.24 04g. of the acid required 8.5c.c. of $\frac{N}{10}$ NaOH and so the mean molecular weight is 282.82 (stearic acid = 284.29).

The saponification products of V consist of ethyl alcohol and a white crystalline mass of fatty acids. 3–5–dinitro–benzoate of the former melts at 91°. The latter was proved to be a mixture of saturated acid of m. p. 62.5° and a small quantity of some unsaturated fatty acids. The mean mol. wt. of the saturated acid is 262.1. (Palmitic acid = 256).

7. Fraction of lower boiling point than 78°.

The fraction has strong pungent smell of acetaldehyde and gives bluish violet coloration characteristic for isovaleraldchyde with vanilline–sulphuric acid reagent.

p–nitroph. prepared melts at 127°. (acetaldehyde)

III. In the case of the saké

77.84 liters were treated in the same way as above.

1. Volatile acid: The fraction of b. p. above 123°C was not obtained.

2. Base: The picrate is yellow needle and melts at 219°C.

3. Fusel oil:

Fract.	b. p.	yield	Fusel oil exist	Ester	3–5–Dinitro-denzoate m. p.	3–5–Dinitro-denzoate N %
I	78—80°	128c.c.	2.5g.	—	—	—
II	80—102	8.2g.	1.1	—	—	—
III	102—113	1.3	0.65	—	55°	11.13 (ethyl and bufyl)
IV	113—123	0.6	—	1.13%	55	10.45 (but.)
V	123—130	5.2	—	1.52	—	—
VI	130—131	1.7	—	—	62	9.91 (amyl)
VII	residue	0.5	Furfurol reaction is positive.			

4. Active amyl alcohol:

α_D^{15} of fusel oil is −1.42°. So the approximate content of secondary butyl carbinol in total amyl alcohol is 21.62%.

5. Oil of high boiling point.

The fraction corresponding to ethylester of higher fatty acids amounts only 6.4g. with addition of distilling residue. Perhaps most part of the esters might have gone to the cake because they were almost insoluble in water or alcohol of 75%.

6. The properties of the fraction of lower boiling point in the same as the case of the moto.

UEBER DIE SCHIMMELPILZE WELCHE SICH BEI DER HERSTELLUNG DES LECKERBISSENS "ONTJOM" UND "TEMPEH" (JAVA UND SUMATRA) MITWIRKEN.

Von

R. Nakazawa u. Y. Takeda.

(Received March 1 st., 1928)

"Ontjom" und "Tempeh" sind die Namen von Nahrungsmitteln welche die Einwohner der genannten Inseln herstellen.

Nach einer Mitteilung von Prinsen Geerligs[1] wird *Rhizopus Oryzae* zu Herstellung von Ontjom aus Erdnussen benutzt, ebenso zu Bereitung von Tempeh auf Soyabonen. Went[2] beobachtet dagegen dass zu Herstellung der genannten Nahrungsmitteln *Monilia sitophila* angewandte wird.

Im Gegensatz zu beiden genannten Authoren haben die Verff. bei ihren Untersuchungen beobachtet dass in Hauptsache *Penicillum brevicaule* bei Bereitung von Ontjom und Tempeh mitwirken, nebenbei wurden kleinen Mengen von *Rhizopus*, *Aspergillus* und *Mucor* identifiziert.

---

(1) Lafer: Handbuch der technischen Mykologie, Bd. **4** S. 494.
(2) Went: Cent. f. Bakt. 2 Abt. 1901, Bd. **7** S. 544.

ON THE PROTEIN OF THE TUBER OF YAM.

II. THE FORM OF THE PROTEIN OF THE TUBER OF YAM.

By

Teizo Takahashi.

(Received May 22nd., 1928)

By the further investigation on the protein of the tuber of yam (Nagaimo), which was believed as a glycoprotein, mucin, the author found that this substance was composed of a mannan and protein. But these two components do not so strongly combined as glycoprotein. By dissolving this substance in 0.2% NaOH solution, only the protein dissolves and the mannan remains undissolved. The amount of the mannan is 12.36% of this substance. By the hydrolysis of this mannan the author obtained pure mannose. The author determined the amount of nitrogen of various forms by the Van Slyke's method and obtained the following results.

Total N	17.33
20% HCl insol. N	0.05
20% HCl sol. N	17.28
Ammonia N	1.04
Humin N	0.22
Diamino N	6.50
Amino N	2.51
Non-amino N	3.99
Arginin N	4.46
Histidin N	0.96
Cystin N	0.05
Lysin N	1.03
Mono-amino N	9.52
Tryptophan	+

Furthermore the author obtained no glucosamin from this substance.

So the author concludes that the substance, which was formerly believed as a glycoprotein, mucin of the tuber of yam, is a special mucilage which is composed of protein and mannan.

昭和三年六月七日印刷
昭和三年六月十日發行

東京帝國大學農學部内
發行兼編輯者　松山芳彦

東京帝國大學農學部内、日本農藝化學會
印刷者　河村秀兼

東京帝國大學農學部内
印刷所　農藝化學教室印刷所